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Inactivation of Avirulent *Yersinia pestis* in Butterfield's Phosphate Buffer and Frankfurters by UVC (254 nm) and Gamma Radiation†

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ABSTRACT

Yersinia pestis is the causative agent of plague. Although rare, pharyngeal plague in humans has been associated with consumption or handling of meat prepared from infected animals. The risks of contracting plague from consumption of deliberately contaminated food are currently unknown. Gamma radiation is a penetrating form of electromagnetic radiation, and UVC radiation is used for decontamination of liquids or food surfaces. Gamma radiation D_{10}-values (the radiation dose needed to inactivate 1 log unit pathogen) were 0.23 (±0.01) and 0.31 (±0.03) kGy for avirulent *Y. pestis* inoculated into Butterfield's phosphate buffer and onto frankfurter surfaces, respectively, at 0°C. A UVC radiation dose of 0.25 J/cm^2 inactivated avirulent *Y. pestis* suspended in Butterfield's phosphate buffer. UVC radiation doses of 0.5 to 4.0 J/cm^2 inactivated 0.97 to 1.20 log units of the *Y. pestis* surface inoculated onto frankfurters. A low gamma radiation dose of 1.6 kGy could provide a 5-log reduction and a UVC radiation dose of 1 to 4 J/cm^2 would provide a 1-log reduction of *Y. pestis* surface inoculated onto frankfurters. *Y. pestis* was capable of growth on frankfurters during refrigerated storage (10°C). Gamma radiation of frankfurters inhibited the growth of *Y. pestis* during refrigerated storage, and UVC radiation delayed the growth of *Y. pestis.*

Pharyngeal plague can be contracted through the handling or consumption of raw or cooked meat products prepared from animals infected with the pathogen *Yersinia pestis (1–3, 6).* The risk of contracting plague, and the associated morbidity and mortality, through the consumption or handling of foods deliberately contaminated with *Y. pestis* are currently unknown. Because *Y. pestis* is listed as a select agent for both food safety and food defense *(21),* the efficacy of nonthermal food processing intervention technologies to inactivate *Y. pestis* in food products should be evaluated.

Gamma radiation and UVC radiation (254 nm) are nonthermal processing technologies that can be used to decontaminate foods or food surfaces. Ionizing radiation can inactivate foodborne pathogens such as *Escherichia coli* O157:H7, *Salmonella, Staphylococcus aureus, Listeria monocytogenes,* and *Y. enterocolitica* on raw and ready-to-eat meat products *(5, 12, 20, 22).* A petition to allow irradiation of ready-to-eat foods is currently pending with the U.S. Food and Drug Administration (FDA) *(8).* UVC is an FDA-approved source of radiation for decontamination of food surfaces *(24).*

Although much information is available on the use of radiation to inactivate common foodborne pathogens *(5, 12, 20, 22),* relatively little information is available on the radiation (gamma or UVC) needed to inactivate *Y. pestis.* The purpose of this study was to determine the ability of *Y. pestis* to survive treatment with gamma or UVC radiation when the pathogen was suspended in buffer or was surface inoculated onto frankfurters that contained the commonly used antimicrobials potassium lactate and sodium diacetate *(10, 23).*

MATERIALS AND METHODS

Frankfurters. Freshly made frankfurters were purchased from a local manufacturer. The frankfurters consisted of beef, pork, water, salt, flavoring, paprika, sodium phosphate, sodium diacetate (0.07%), potassium lactate (1.13%), sodium erythorbate, and sodium nitrate. The antimicrobials sodium diacetate and potassium lactate are commonly used in commercially available frankfurters to inhibit the growth of *L. monocytogenes.* Frankfurters were frozen at −20°C and gamma irradiated at a dose of 10 kGy to eliminate background microflora.

Y. pestis. Four avirulent *Y. pestis* strains (KUMA, Yokohama, KIM5, and CO99) were obtained from Dr. Susan Straley (University of Kentucky, Lexington) and Dr. Robert Brubaker (Michigan State University, East Lansing) through Dr. George Paoli at the U.S. Department of Agriculture Eastern Regional Research Center (Wyndmoor, PA). The *Y. pestis* strains were propagated on brain heart infusion (BHI) agar (Difco, Becton Dickinson, Sparks, MD) at 30°C for approximately 3 days and maintained at 0 to 4°C until ready for use. Sommers and Novak *(18)* previously determined that there is no virulence factor–dependent difference in the radiation resistance between virulent and avirulent forms of the closely related bacterium *Yersinia enterocolitica* when sus-

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TABLE 1. *Gamma radiation inactivation of* Yersinia pestis *suspended in Butterfield's phosphate buffer or surface inoculated onto frankfurters*

	Log reduction after irradiation at[a]:				
Culture type	0 kGy	0.25 kGy	0.50 kGy	0.75 kGy	1.0 kGy
Buffer	0.00 (±0.00)	1.01 (±0.05)	2.07 (±0.03)	3.10 (±0.02)	4.21 (±0.04)
Frankfurter	0.00 (±0.00)	0.99 (±0.13)	1.76 (±0.18)	2.33 (±0.22)	3.38 (±0.03)

[a] Log reductions are the mean of three independent experiments, with the standard error of the mean shown in parentheses.

pended in buffer or raw ground pork or of the closely related *Yersinia pseudotuberculosis* (unpublished data). Therefore, avirulent *Y. pestis* strains KUMA, Yokohama, KIM5, and CO99 were used in this study.

Propagation and inoculation. Each *Y. pestis* strain was cultured independently in 30 ml of BHI broth (Difco, Becton Dickinson) in a sterile 50-ml conical tube at 37°C (at 150 rpm) for 18 h to a density of approximately 10^7 CFU/ml in a model G24 incubator (New Brunswick Scientific, Edison, NJ). The cultures were then sedimented by centrifugation at 5,000 rpm for 5 min (4°C) in a Marathon 21000R centrifuge (Fisher Scientific, Needham Heights, MA). The cell pellets were then resuspended in Butterfield's phosphate buffer (BPB; Applied Research Institute, Newtown, CT) as a cocktail.

The *Y. pestis* cocktail in BPB (5 ml) was then pipetted into sterile borosilicate glass test tubes (16 by 150 mm; Fisher Scientific) for gamma radiation or into sterile polystyrene petri dishes (10 by 15 mm; Fisher Scientific) for UVC radiation. The depth of the *Y. pestis* suspension in BPB was 2 to 3 mm in the bottom of the petri dishes. Individual frankfurters were thoroughly rolled in 0.2 ml of *Y. pestis* that was pipetted onto the surface of a sterile polynylon bag (stock no. 7554, Uline, Inc., Philadelphia, PA) and vacuum packed with a model A300 vacuum packer (Multivac, Kansas City, MO) in sterile polynylon bags to 30 mB before gamma radiation. For UVC radiation, individual inoculated frankfurters were exposed to UVC radiation before packaging, as would be done on a conveyor in a frankfurter manufacturing facility.

The same procedures for inoculation and recovery and enumeration of *Y. pestis* on frankfurters was used for determination of growth potential (8 weeks at 10°C), except that frankfurters were sealed in gas- and moisture-impermeable polynylon bags (Uline). Cell densities were adjusted by dilution in BPB to achieve starting levels of approximately 10^3 CFU/g for the growth potential experiments.

Gamma radiation. A self-contained ^{137}Cs irradiator (Lockheed Georgia Company, Marietta, GA) at a dose rate of 0.086 kGy/min was used for all exposures *(19).* The radiation source consisted of 23 individually sealed source pencils in an annular array. The cylindrical sample chamber (22.9 by 63.5 cm) was located central to the array when placed in the operating position. Inoculated samples were placed vertically and centrally in the sample chamber in a 4-mm-thick polypropylene bucket to ensure a high dose uniformity ratio (<1.1:1.0). The temperature during irradiation (0°C) was monitored by thermocouple and maintained by introduction of the gas phase from a liquid nitrogen source directly into the top of the sample chamber. The absorbed dose was verified with temperature-tempered 5-mm alanine pellets that were then analyzed with an EMS 104 EPR analyzer (Bruker, Billerica, MA). The absorbed doses were typically within 5% of target values.

UVC radiation. The custom-made UVC irradiator contained four 24-in. (61-cm) UVC emitting bulbs (Atlantic Ultraviolet, White Plains, NY). The apparatus delivered a UVC dose of 10 mW/cm^2/s as determined with a calibrated UVX Radiometer (UVP, Inc., Upland, CA) at a distance of 20 cm from the bulbs. A dose of 1 J/cm^2 is equal to 1 W/cm^2/s. A UVC dose of 1 J/cm^2 was therefore obtained by exposure to the UVC source for 100 s. Because frankfurters have an overall cylindrical shape, single frankfurters were irradiated by rotating them 90° four times during the exposure to UVC radiation. Therefore, a frankfurter exposed to 4 J/cm^2 UVC radiation received four 1.0-J/cm^2 exposures. UVC treatment did not increase the surface temperature of the frankfurters to greater than 20°C, as determined using a hand-held infrared thermometer (model 25625-40, Oaklon, Inc., Vernon Hills, IL).

Sample dilution and plating. After irradiation, the samples were assayed for surviving bacteria with a standard pour plate procedure. *Y. pestis* irradiated in BPB were further serially diluted in BPB *(17, 19).* For *Y. pestis* on frankfurters, a surface-rinse procedure was used *(19).* Approximately 100 ml of BPB was added to the sample bags containing the frankfurters. The samples were then mixed by surface wash for 2 min, and decimal serial dilutions were made in BPB. One milliliter of appropriately diluted samples was then placed in sterile polystyrene petri plates, and 20 to 25 ml of BHI agar (45°C) was added. The petri plates (two per dilution) containing the *Y. pestis* were then incubated at 30°C for 3 days before colonies were counted.

***D_{10}*-values and log reductions.** The mean plate counts of the treated samples (N) were divided by the average control plate counts (N_0) to give a survivor ratio (N/N_0). The log(N/N_0) of values were then used for determination of D_{10}-values and other sta-

TABLE 2. *UVC light inactivation of* Yersinia pestis *suspended in Butterfield's phosphate buffer or surface inoculated onto frankfurters*

	Log reduction after irradiation at[a]:						
Culture type	0 J/cm^2	0.13 J/cm^2	0.25 J/cm^2	0.5 J/cm^2	1.0 J/cm^2	2.0 J/cm^2	4.0 J/cm^2
Buffer	0.00 (±0.00)	3.81 (±0.55)	ND	ND	ND		
Frankfurter	0.00 (±0.00)			0.97 (±0.08)	1.01 (±0.07)	1.10 (±0.06)	1.20 (±0.41)

[a] Log reductions are the mean of three independent experiments, with the standard error of the mean shown in parentheses. ND, not detected.

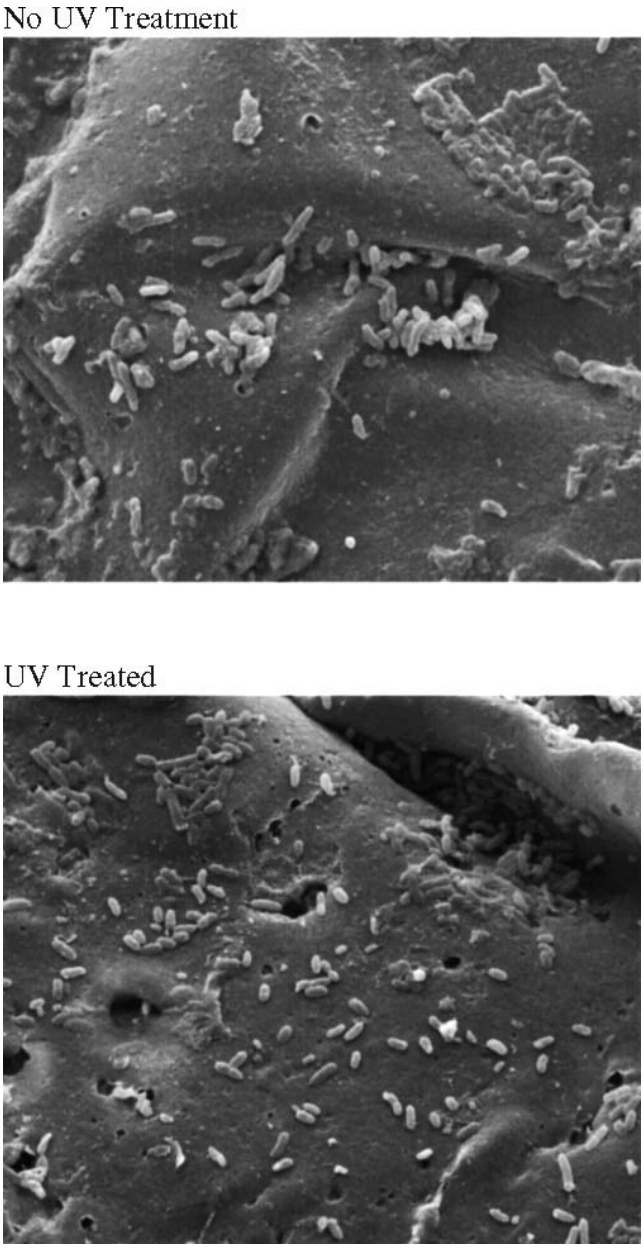

FIGURE 1. *Scanning electron microscopy of* Y. pestis *surface inoculated onto frankfurters and treated with UVC radiation.*

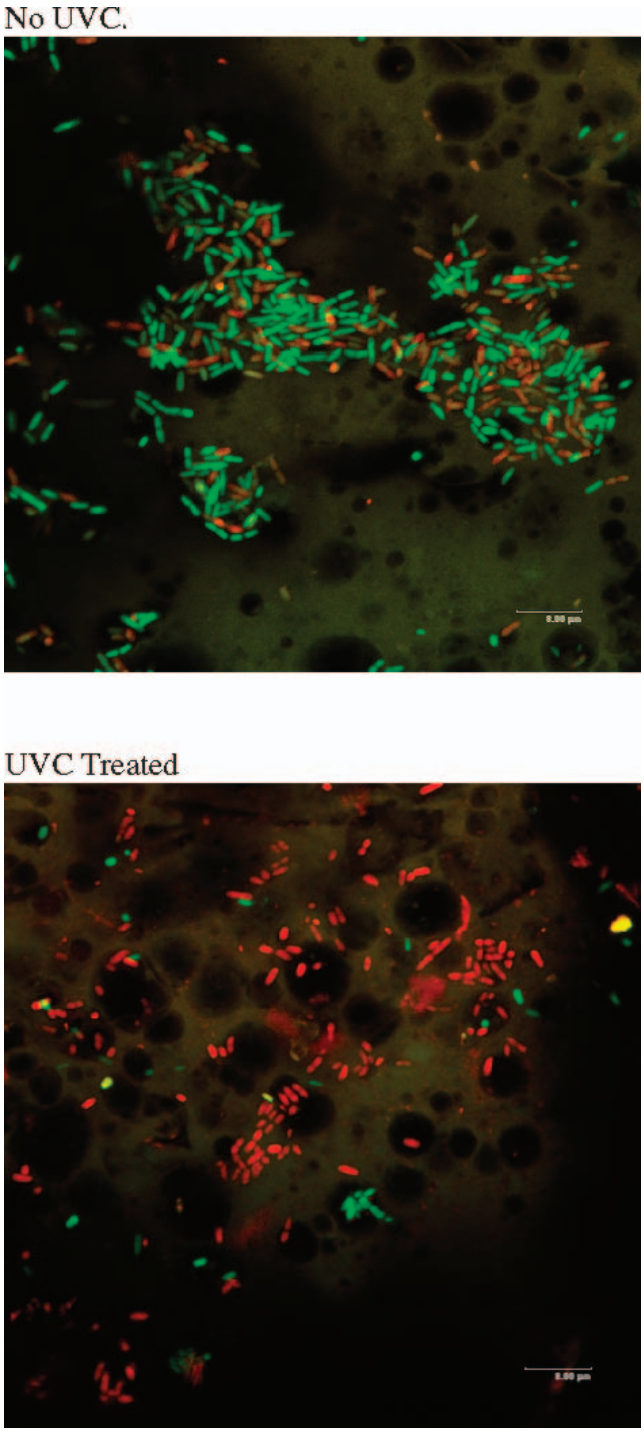

FIGURE 2. *Confocal laser scanning fluorescence microscopy of live (green) and dead (red) stained* Y. pestis *surface inoculated onto frankfurters and treated with UVC radiation.*

tistical analyses. D_{10}-values were determined from the reciprocal of the slope following linear regression as determined by least-squares analysis *(4).*

Statistical analysis. Each experiment was conducted independently three times. Statistical analysis functions of MS Excel (Microsoft Corp., Redmond, WA) were used for routine calculations, descriptive statistics, and analyses of variance.

Scanning electron microscopy. Frankfurters were inoculated and UVC irradiated as described previously. Thin (1 mm) 1-cm slices of the surfaces of frankfurter samples were excised with a stainless steel razor blade and immersed in ~20 ml of a 2.5% glutaraldehyde–0.1 M imidazole buffer solution (pH 7.2). The samples were then stored for a few days in sealed vials. For sample processing, slices were washed in imidazole buffer and then dehydrated by exchange with 20-ml volumes of graded ethanol solutions (50, 80, and 100%), with two changes at each concentration. Samples were then critical point dried with liquid CO_2, and dry slices were glued with Duco cement (ITW Performance Polymers, Riviera, FL) and colloidal silver adhesive (Electron Microscopy Sciences, Hatfield, PA) to specimen stubs. The mounted samples were sputter coated with gold, and digital images were captured with a model Quanta 200 FEG scanning electron microscope (FEI Co., Inc., Hillsboro, OR) operated in the high-vacuum secondary electron imaging mode (10 kV).

Confocal laser scanning fluorescence microscopy. Frankfurters were inoculated and UVC irradiated as described previously. Thin (2 to 3 mm) slices of the surface of frankfurter sam-

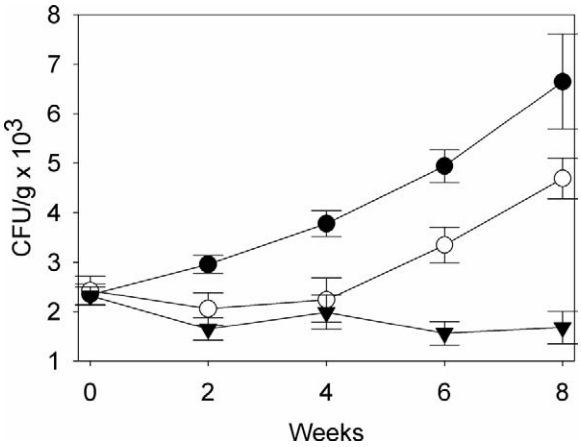

FIGURE 3. *Growth of* Y. pestis *on frankfurters (10°C): untreated controls (closed circle) and samples exposed to UVC (open circle) and gamma (triangle) radiation.*

ples were immersed in 1 ml of the nucleic acid staining viability dye kit L-7012 (Molecular Probes, Eugene, OR) (3 μl of A + B components per ml of imidazole buffer solution). After 20 min of incubation in the dye solution, concentrated glutaraldehyde was added to a final concentration of 2.5%, samples were transferred into 50 μl of staining solution in a glass-bottom microwell dish (MatTek Corp., Ashland, MA), and surfaces images were captured with a model IRBE optical microscope and a 63× water immersion lens coupled to a TCS-SP confocal fluorescence attachment (Leica Microsystems, Exton, PA). Fluorescence was excited by the 488-nm line of an Argon laser, and emission data were collected simultaneously as stacks of optical sections into two channels, 500 to 540 nm for glutaraldehyde-induced autofluorescence (marking the superficial matrix of the frankfurter and fluorescence from the viability dye, Syto 9, labeling the microbial cells) and 640 to 680 nm for the ''nonviable'' dye, propidium iodide. Maximum projection images of the two stacks of optical sections in the two channels were overlaid and compared to determine the abundance of viable and nonviable cells on the convoluted surfaces of treated and nontreated samples.

RESULTS AND DISCUSSION

Gamma radiation, emitted as photons from cobalt-60 or cesium-137, is a penetrating form of electromagnetic radiation that can be used to inactivate foodborne pathogens either in or on meat products. Gamma radiation exerts its effects directly by induction of single- and double-strand DNA breaks and indirectly by oxidative DNA damage through the radiolysis of water *(11, 15).* In this study, the mean (±standard error of the mean) D_{10}-values (the radiation dose needed to inactivate 90% of a foodborne pathogen) were 0.23 (±0.01) kGy (R^2 = 0.99) for *Y. pestis* suspended in buffer and 0.31 (±0.01) kGy (R^2 = 0.96) for *Y. pestis* inoculated onto frankfurter surfaces (Table 1). Gamma radiation D_{10}-values for foodborne pathogens are typically lower in aqueous solutions because of greater exposure to the radiolysis products of water as opposed to suspension in meat products, where other organic molecules interact with free radicals *(11, 15).*

In previous studies, the gamma radiation D_{10}-values for the common foodborne pathogens *Salmonella, L. monocytogenes, S. aureus, E. coli* O157:H7, and *Y. enterocolitica* were 0.61, 0.54, 0.47, 0.36, and 0.15 kGy, respectively, when inoculated onto complex ready-to-eat foods, including frankfurters *(19).* The D_{10}-values for *Salmonella, L. monocytogenes, S. aureus,* and *E. coli* O157:H7 suspended in refrigerated (4°C) raw ground pork were 0.51, 0.48, 0.43, and 0.30 kGy, respectively *(22).* The D_{10}-value of *Y. enterocolitica* suspended in raw ground pork was 0.19 to 0.23 kGy *(11, 17, 18).* Sommers and Niemira *(16)* found that the gamma radiation D_{10}-value of *Y. pestis* suspended in refrigerated (0°C) raw ground pork was 0.27 kGy. Therefore, *Y. pestis* is one of the more gamma-radiation-sensitive foodborne pathogens and could easily be inactivated on meat and ready-to-eat meat products by ionizing radiation.

UVC radiation is a nonpenetrating form of electromagnetic radiation that is used to inactivate microorganisms in solutions and on food surfaces *(5, 9, 20, 25).* It exerts its effects through creation of cyclobutane pyrimidine dimers and 6-4 photoproducts in chromosomes that interfere with the DNA replication process *(7).* There is relatively little information on the resistances of common foodborne pathogens on the surfaces of raw meat and ready-to-eat meat products exposed to UVC radiation; however, one researcher listed four pathogens in order of relative resistance: *L. monocytogenes* > *S. aureus* > *Salmonella* > *E. coli (5). Y. pestis* was inactivated in BPB at a UVC dose of 0.25 J/cm^2 (Table 2). When *Y. pestis* was surface inoculated into frankfurters, UVC doses of 0.5 to 4 J/cm^2 resulted in 0.97- to 1.2-log reductions (greater than 90% of the pathogen). In a recent study using the same methodology and UVC irradiation apparatus, a UVC radiation dose of 4 J/cm^2 inactivated approximately 2.0 log CFU of *L. monocytogenes* on frankfurter surfaces *(13).* Although the surface of the frankfurters used in this study were relatively smooth in comparison to other ready-to-eat meat products such as turkey ham *(13),* scanning electron microscopy revealed many places where the bacterium could be shielded from UVC radiation (Fig. 1). The survival of the bacterium on frankfurter surfaces, as examined with a confocal laser scanning fluorescence microscope on samples stained with live-dead stains, is shown in Figure 2.

When *Y. pestis* was inoculated onto frankfurters and growth was assessed during an 8-week storage period (at 10°C), *Y. pestis* proliferated modestly (Fig. 3). UVC radiation delayed the growth of the pathogen for approximately 4 weeks, but gamma radiation completely inhibited growth of the survivors for 8 weeks.

Neither gamma nor UVC radiation had a negative impact on the quality (color and texture) of frankfurters or bologna that contained potassium lactate or sodium diacetate *(13, 14). Y. pestis* is very sensitive to gamma radiation in comparison to other common vegetative foodborne pathogens and could easily be inactivated in either aqueous solution or on frankfurters. *Y. pestis* was very sensitive to UVC radiation when suspended in BPB but was more resistant to UVC radiation than was *L. monocytogenes* when inoculated onto the surface of frankfurters.

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